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RESEARCH PAPER

Estimation of defence related compounds in healthy and malformed vegetative tissues of mango in five states of northern India induced in response to low temperature and high relative humidity

V. RANI* AND G. BAINS
Department of Plant Physiology, College of Basic Science and Humanities, G.B. Pant University of Agriculture and Technology, PANTNAGAR (UTTARAKHAND) INDIA

Abstract : Mango malformation disease occurs as the result of accumulation of ethylene under stress in plant parts following infection with *Fusarium mangiferae.* In response to fungal infection several defence related compounds like total phenols, polyphenol oxidase and lipooxygenase accumulate in plant parts. In malformed tissue samples of five commercial mango varieties collected from different states recorded an increase in total phenol content and a reduced polyphenol oxidase and lipoxygenase activity.

Key Words : Mango malformation, Total phenols, Polyphenol oxidase, Lipooxygenase, Ethylene, *Fusarium mangiferae*

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INTRODUCTION

Mango holds a place of pre-eminence amongst the major tropical fruit crops and India is a major producer and chief exporter of mango in the world. Mango suffers from several diseases and disorders during its life cycle. Among the known diseases of mango, malformation is considered as the most destructive disease as it causes heavy economic losses of about 50-80 per cent on global basis (NHB, Indian Horticulture Database, 2011).

The malady appears in two forms *viz.*, vegetative and floral. In vegetative malformation, the seedlings produce small shootlets bearing a cluster of small scaly leaves. Apical dominance is lost in affected seedlings. The multi-branching of shoot apex with scaly leaves is known as Bunchy Top or Witch's Broom (Bhatnagar and Beniwal, 1977). In floral malformation rachis is thick, shortened and highly branched with large number of flowers. The flowers are mostly male and rarely bisexual .In malformed panicles flowers are sterile and thus, fruit set is affected, leading to yield loss (Schlosser, 1971).

Miscellaneous etiologies have been proposed for the malady *viz.,* Fungi (Crespo and Cazorla, 2012), mites

*** Author for correspondence:**

(Summanwar *et al.*, 1966), viruses (Sattar, 1946) and physiological factors (Pant, 2000), but stress ethylene produced from the fungal pathogen has been the most credited. Fungal infection is known to elicit the production of defence related compounds in the plant under low temperature and high relative humidity. The present study was, therefore, undertaken to estimate the amount and activity of phenolics etc. under low temperature and high relative humidity condition, which in turn is assumed to cause fungal infection and production of stress ethylene in the mango plant.

MATERIAL AND METHODS

Collection of experimental material :

Five varieties of mango namely Amrapali, Dasheri, Langra, Chausa and Bombay green were selected. The experimental material used was healthy and malformed leaf tissues and was collected from mango orchards of Patna, Bihar, Ranchi, Jharkhand; Allahabad, Uttar Pradesh; Pantnagar, Uttarakhand and New Delhi.

Estimation of total phenol content :

Overall phenol content in leaves was estimated by the method described by Bray and Thorpe (1954). One g fresh leaf tissue was homogenized using mortar and pestle with the addition of 80 per cent ethyl alcohol (v/v). It was then centrifuged at 10,000 rpm for 20 min and the supernatant was filtered with Whatman filter paper number 42. The residue was extracted with 80 per cent ethyl alcohol for five times. The collected supernatants were dried through evaporation in a water bath at 68°C. Residue was put in 10 ml of distilled water. Out of this, 0.1 ml volume was drawn and general volume was made upto 3 ml by adding double distilled water. Freshly made 0.5 ml Folin –Ciocalteau reagent was then added to it. After 3 min of addition of the reagent, 2 ml of 20 per cent carbonate of sodium was mixed in every tube, mixed thoroughly and placed on a warm water bath at 58^0C for 1 min.Then it was cooled to room temperature and absorbance was recorded towards blank at 750 nm with the help spectrophotometer. The values had been expressed as milligram phenols per 100 g leaf tissue.

Estimation of polyphenol oxidase activity:

Extraction and analysis was accomplished using the technique given by Augustin *et al*. (1985). One gram of clean leaf tissue was homogenized in a pre chilled mortar. 10 ml of chilled extraction medium was used and it included phosphate buffer at pH 6.8, 0.2ml, PVP (15 mg/g) and triton X-100 (5 µg/g). Extracted reaction mixture was poured into chilled centrifuge tubes. After that centrifugation was done at 11,000 rpm for 20 min at 20^0C.The particles which were present in the supernatant were filtered with cotton. The pellet was then removed. The supernatant was used for assay after dialyzing at 4^0 C in opposition to 0.2 M phosphate buffer for two days with 2 modifications of buffer. For assay, one ml of 0.05M catechol, various amount of enzyme extract and 0.2 M phosphate buffer pH 6.8 was added to the reaction mixture. At last the enzyme extract was added to initiate the reaction. Absorbance was measured at 410 nm in opposition to the blank after 30 seconds upto three min. The absorbance was recorded between 30 seconds of incubation and the activity of enzyme was formulated from linear part of the curve.

Estimation of lipooxygenase enzyme :

Potassium di-hydrogen phosphate (0.1M) and di-potassium hydrogen phosphate (0.1M) was mixed together in 16:84 ratio and the pH was adjusted to 7.5. For the extraction of enzyme 0.186 g of ethylene diamine tetra acetic acid (EDTA) (0.5M) was added to 100 ml of the reaction mixture.

The activity of lipooxygenase was estimated following the technique of Dodderer *et al*. (1992). The substrate was prepared with the addition of 35 micro litre linoleic acid to 5 ml distilled water containing fifty micro litre tween 20. The pH of reaction mixture was adjusted to 9.0 with 0.2 M sodium hydroxide until the linoleic acid was dissolved and pH remained unchanged. The pH was adjusted to 6.5 by adding 0.2M hydrogen chloride and after adjusting the pH, 0.1M phosphate buffer (PH 6.5) was added to a volume of 100 ml. To determine the lipooxygenase activity, 50 micro litre of the sample was added to 2.9 millilitre substrate. It was then incubated for 10 min after which absorbance was recorded at 234 nm. The protein was estimated by the method given by Lowry *et al*. (1951).

The data were analysed statistically by two factorial Randomized Block Design (RBD).

RESULTS AND DISCUSSION

The results obtained from the present investigation as well as relevant discussion have been summarized under following heads :

Total phenol content (mg phenols/ 100 g) in malformed and healthy leaf tissue samples :

The total phenol content in healthy and malformed tissues was estimated over two months (February-March) during flower initiation to flowering period in Amrapali, Dasheri, Langra, Chausa and Bombay Green

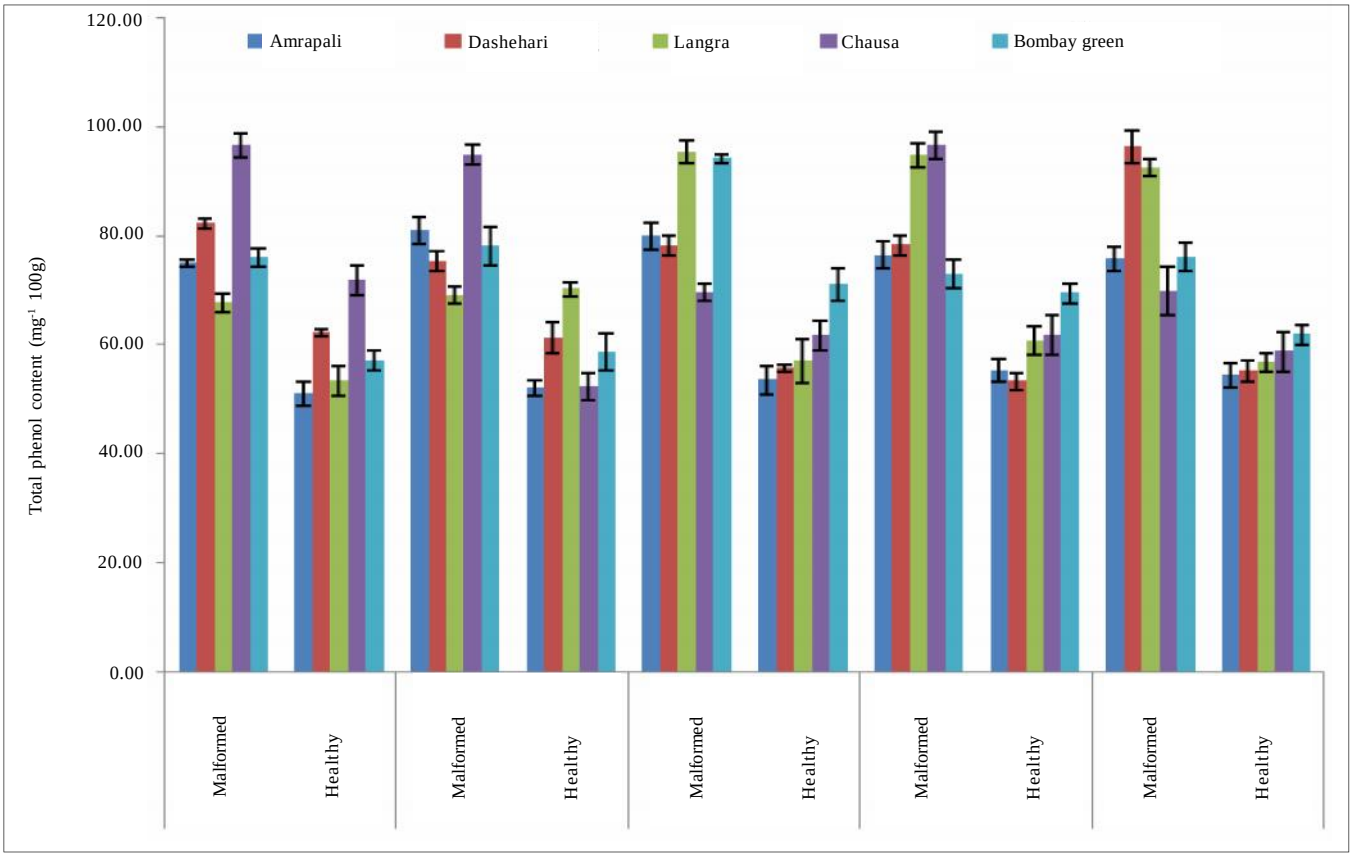

Fig. 1 : Total phenol content (mg/ 100 g) in malformed and healthy leaves of different mango varieties from different states

Table 1: Total phenol content (mg phenols/ 100 g) in malformed and healthy leaf tissues of different mango varieties from different state

Sr. No.	Varieties	Total phenol content (mg^{-1} 100 g)									
		Bihar		Jharkhand		Uttar Pradesh		Uttarakhand		Delhi	
		Malformed	Healthy	Malformed	Healthy	Malformed	Healthy	Malformed	Healthy	Malformed	Healthy
1.	Amrapali	75.00 ±0.67	51.00 ±2.20	81.00 ±2.46	52.00 ±1.36	79.80 ±2.46	53.50 ±2.52	76.38 ±2.46	55.30 ±2.01	75.80 ±2.20	54.30 ±2.20
2.	Dasheri	82.25 ±0.94	62.23 ±0.63	75.25 ±1.83	61.24 ±2.86	78.15 ±1.83	55.75 ±0.63	78.25 ±1.83	53.25 ±1.57	66.28 ±4.52	55.25 ±1.97
3.	Langra	67.60 ±1.65	53.25 ±2.72	69.10 ±1.65	70.16 ±1.34	95.28 ±2.09	56.93 ±4.02	94.68 ±2.26	60.75 ±2.68	92.47 ±1.56	56.75 ±1.78
4.	Chausa	96.57 ±2.29	71.80 ±2.73	94.75 ±1.79	52.25 ±2.41	69.50 ±1.59	61.72 ±2.73	96.50 ±2.48	61.82 ±3.62	69.88 ±4.41	58.73 ±3.62
5.	Bombay green	76.00 ±1.74	57.00 ±1.84	78.00 ±3.53	58.60 ±3.44	94.10 ±0.85	70.96 ±3.00	69.45 ±2.64	72.96 ±1.83	76.02 ±2.64	61.85 ±1.84
	Mean	79.48	59.06	79.62	58.85	83.37	59.77	83.05	60.82	82.09	57.38
		Condition (A)	Variety (B)	Condition (A)	Variety (B)	Condition (A)	Variety (B)	Condition (A)	Variety (B)	Condition (A)	Variety (B)
	S.E.±	0.57	0.90	0.56	0.89	0.61	0.97	0.73	1.16	1.08	1.72
	C.D. (P=0.05)	1.69	2.67	1.68	2.66	1.83	2.90	2.18	3.46	3.23	5.11

cultivars of mango in different states (Table 1 and Fig. 1).

As may be observed, the total phenol content in malformed tissue was higher as compared to healthy tissue in all the cultivars in different states. Total phenol content in healthy vegetative tissue of Amrapali was 51.00, 52.00, 53.50, 55.30 and 54.30 mg phenol/ 100 g of leaf tissues in Bihar, Jharkhand, Uttar Pradesh, Uttarakhand and Delhi, respectively and that in malformed vegetative tissue was 75.00, 81.00, 79.80, 76.38, 75.80 mg phenol/ 100 g of leaf tissues in Bihar, Jharkhand, Uttar Pradesh, Uttarakhand and Delhi, respectively.

Similarly in the healthy vegetative tissue of cultivar, Dasheri, it was found to be 62.23, 61.24, 55.75, 53.25 and 55.25 mg phenol/100 g of leaf tissues in Bihar, Jharkhand, Uttar Pradesh, Uttarakhand and Delhi, respectively and that in malformed vegetative tissue was 96.57, 94.75, 69.50, 96.50 and 69.86 mg phenol/ 100 g of leaf tissues in Bihar, Jharkhand, Uttar Pradesh, Uttarakhand and Delhi, respectively

In the healthy vegetative tissue of Langra it was 53.25, 70.16, 56.93, 60.75 and 56.75 mg phenol/100 g of leaf tissues in Bihar, Jharkhand, Uttar Pradesh, Uttarakhand and Delhi, respectively and that in malformed vegetative tissue was 67.60, 69.10, 95.28, 94.68 and 92.47 mg phenol/ 100 g of leaf tissues in Bihar, Jharkhand, Uttar Pradesh, Uttarakhand and Delhi, respectively.

Similarly, in the healthy vegetative tissue of cultivar, Chausa it was found to be 71.80, 52.25, 61.72, 61.82 and 58.73 mg phenol/100 g of leaf tissues in Bihar, Jharkhand, Uttar Pradesh, Uttarakhand and Delhi, respectively and that in malformed vegetative tissue was 96.57, 94.75, 69.50, 96.50 and 69.86 mg phenol/ 100 g of leaf tissues in Bihar, Jharkhand, Uttar Pradesh, Uttarakhand and Delhi, respectively .

In the healthy vegetative tissue of Bombay green it was 57.00, 58.60, 70.96, 72.96 and 61.85 mg phenol/ 100 g of leaf tissues in Bihar, Jharkhand, Uttar Pradesh, Uttarakhand and Delhi, respectively and that in malformed vegetative tissue was 76.00, 78.00, 94.10, 69.45 and 76.02 mg phenol/ 100 g of leaf tissues in Bihar, Jharkhand, Uttar Pradesh, Uttarakhand and Delhi, respectively.

In a similar study, chilling stress and water deficit condition lead to increase in total phenolic content in *Rehmannia glutinosa* (Chung *et al.*, 2006). But in some other findings opposite effect was observed. In stressed sample lower phenolic content was observed as compared to control ones (Weidner *et al*., 2009). In another experiment there was no major effect on the total phenolic compounds in pea roots when exposed to chilling stress, but it significantly decreased the content of flavones (Rudikowskaya *et al*., 2008). Under abiotic stress condition the production of phenolic compounds in plant tissue rises (Weidner *et al*., 2009). Comparable consequences were recorded in earlier researches on seedlings of *V. vinifera* exposed to chilling stress.

Polyphenol oxidase activity (min^{-1} g^{-1} fresh weight) in malformed and healthy leaf tissues of different mango varieties from different states :

Polyphenol oxidase activity in healthy and malformed tissues was estimated over two months (February-March) during flowering initiation to flowering period in Amrapali, Dushehri, Langra, Chausa and Bombay Green cultivars of mango (Table 2 and Fig. 2)

As may be observed (Table 2 and Fig. 2) polyphenol oxidase activity in malformed tissue was lower as compared to healthy tissue in all the cultivars in different states. Poly phenol oxidase activity in healthy vegetative tissue of Amrapali was 8.39 , 8.32 , 8.31 , 8.34 and 8.34 $min^{-1}g^{-1}$ fresh weight of leaf tissues in Bihar, Jharkhand, Uttar Pradesh, Uttarakhand and Delhi, respectively and that in malformed vegetative tissue was 5.32, 6.31, 6.12, 5.52 and 5.25 $min^{-1}g^{-1}$ fresh weight of leaf tissues in Bihar, Jharkhand, Uttar Pradesh, Uttarakhand and Delhi, respectively.

Similarly in the healthy vegetative tissue of cultivar, Dasheri, it was found to be 4.26, 4.21, 4.75, 8.71 and 8.60 min^{-1} g^{-1}fw of leaf tissues in Bihar, Jharkhand, Uttar Pradesh, Uttarakhand and Delhi, respectively and that in malformed vegetative tissue was 2.99, 3.97, 3.75, 4.57 and 4.52 min^{-1} g^{-1}fw of leaf tissues in Bihar, Jharkhand, Uttar Pradesh, Uttarakhand and Delhi, respectively.

Similarly in the healthy vegetative tissue of cultivar Langra it was found to be 5.32, 6.11, 5.20, 5.20 and 5.00 $min^{-1}g^{-1}$ fresh weight of leaf tissues in Bihar, Jharkhand, Uttar Pradesh, Uttarakhand and Delhi, respectively and that in malformed vegetative tissue was 3.01, 3.12, 4.25, 4.92 and 4.56 $min^{-1}g^{-1}$ fresh weight of leaf tissues in Bihar, Jharkhand, Uttar Pradesh, Uttarakhand and Delhi, respectively .

In the healthy vegetative tissue of cultivar, Chausa, it was found to be 4.20,4.25,6.27,6.28 and 5.98 $min^{-1}g^{-1}$

fresh weight of leaf tissues in Bihar, Jharkhand, Uttar Pradesh, Uttarakhand and Delhi, respectively and that in malformed vegetative tissue was 3.11, 4.11, 4.92, 3.75 and 3.57 $min^{-1}g^{-1}$ fresh weight of leaf tissues in Bihar, Jharkhand, Uttar Pradesh, Uttarakhand and Delhi, respectively .

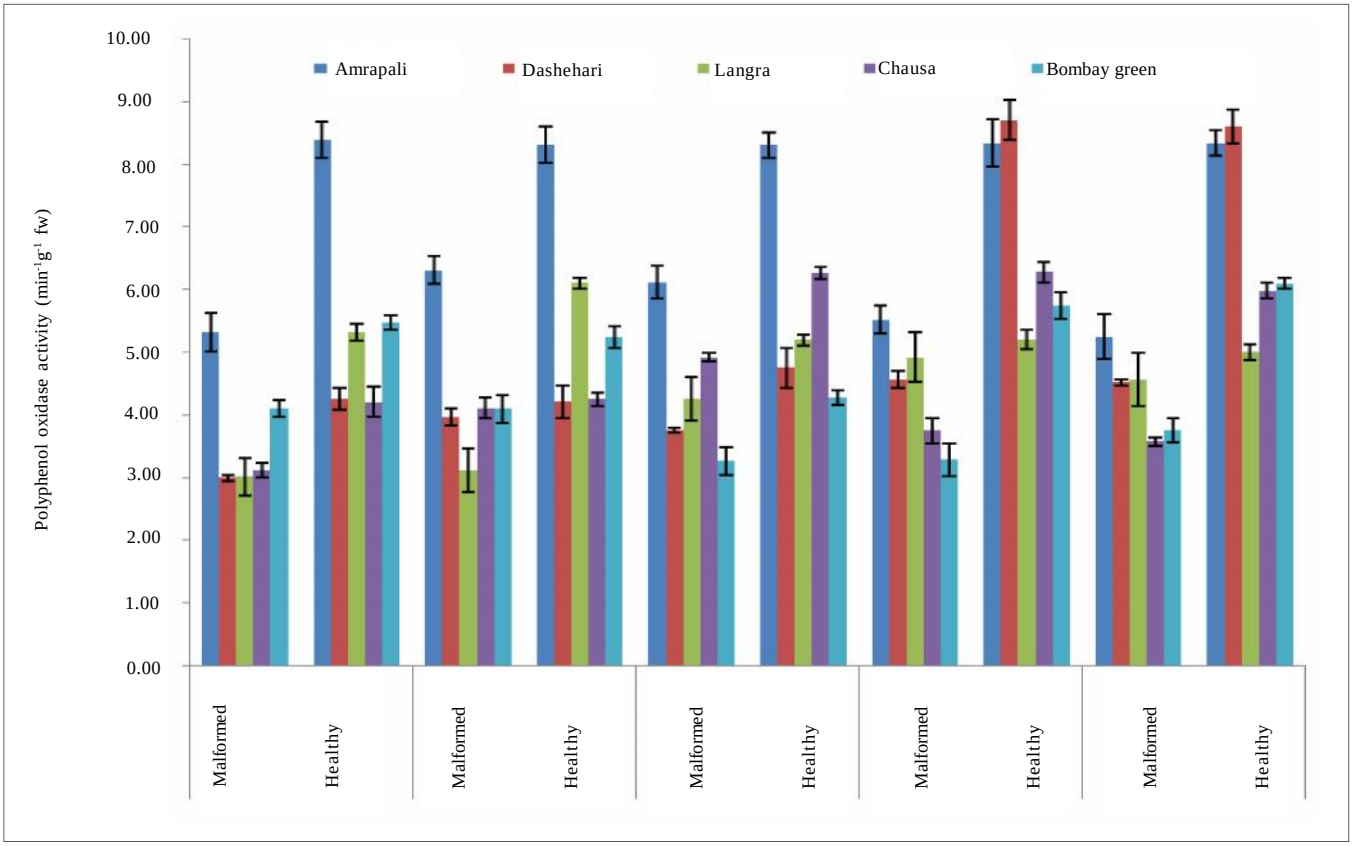

Fig. 2 : Polyphenol oxidase (min^{-1} g^{-1} fw) in malformed and healthy leaves of different mango varieties from different states

Table 2 : Polyphenol oxidase activity (min^{-1} g^{-1}fw) in malformed and healthy leaves of different mango varieties from different states

Sr. No.	Variety	Polyphenol oxidase activity (min^{-1} g^{-1}fw)									
		Bihar		Jharkhand		Uttar Pradesh		Uttarakhand		Delhi	
		Malformed	Healthy	Malformed	Healthy	Malformed	Healthy	Malformed	Healthy	Malformed	Healthy
1.	Amrapali	5.32 ±0.31	8.39 ±0.29	6.31 ±0.22	8.32 ±0.29	6.12 ±0.27	8.31 ±0.20	5.52 ±0.22	8.34 ±0.38	5.25 ±0.36	8.34 ±0.20
2.	Dasheri	2.99 ±0.04	4.26 ±0.18	3.97 ±0.13	4.21 ±0.27	3.75 ±0.04	4.75 ±0.31	4.57 ±0.13	8.71 ±0.31	4.52 ±0.04	8.60 ±0.27
3.	Langra	3.01 ±0.30	5.32 ±0.13	3.12 ±0.35	6.11 ±0.08	4.25 ±0.35	5.20 ±0.08	4.92 ±0.39	5.20 ±0.15	4.56 ±0.42	5.00 ±0.13
4.	Chausa	3.11 ±0.11	4.20 ±0.24	4.11 ±0.16	4.25 ±0.11	4.92 ±0.07	6.27 ±0.09	3.75 ±0.20	6.28 ±0.16	3.57 ±0.07	5.98 ±0.13
5.	Bombay Green	4.10 ±0.13	5.47 ±0.12	4.10 ±0.22	5.25 ±0.17	3.26 ±0.22	4.28 ±0.12	3.28 ±0.27	5.75 ±0.21	3.75 ±0.19	6.10 ±0.09
	Mean	3.71	5.53	4.32	5.63	4.46	5.76	4.41	6.86	4.33	6.80
		Condition (A)	Variety (B)	Condition (A)	Variety (B)	Condition (A)	Variety (B)	Condition (A)	Variety (B)	Condition (A)	Variety (B)
	S.E.±	0.05	0.08	0.05	0.08	0.06	0.09	0.05	0.09	0.07	0.12
	C.D. (P=0.05)	0.16	0.26	0.15	0.24	0.18	0.29	0.17	0.27	0.22	0.36

In the healthy vegetative tissue of cultivar, Bombay green, it was found to be 5.47, 5.25, 4.28, 5.75 and 6.10 $min^{-1}g^{-1}$ fresh weight of leaf tissues in Bihar, Jharkhand, Uttar Pradesh, Uttarakhand and Delhi, respectively and that in malformed vegetative tissue was 4.10, 4.10, 3.26, 3.28 and 3.75 $min^{-1}g^{-1}$ fresh weight of leaf tissues in Bihar,

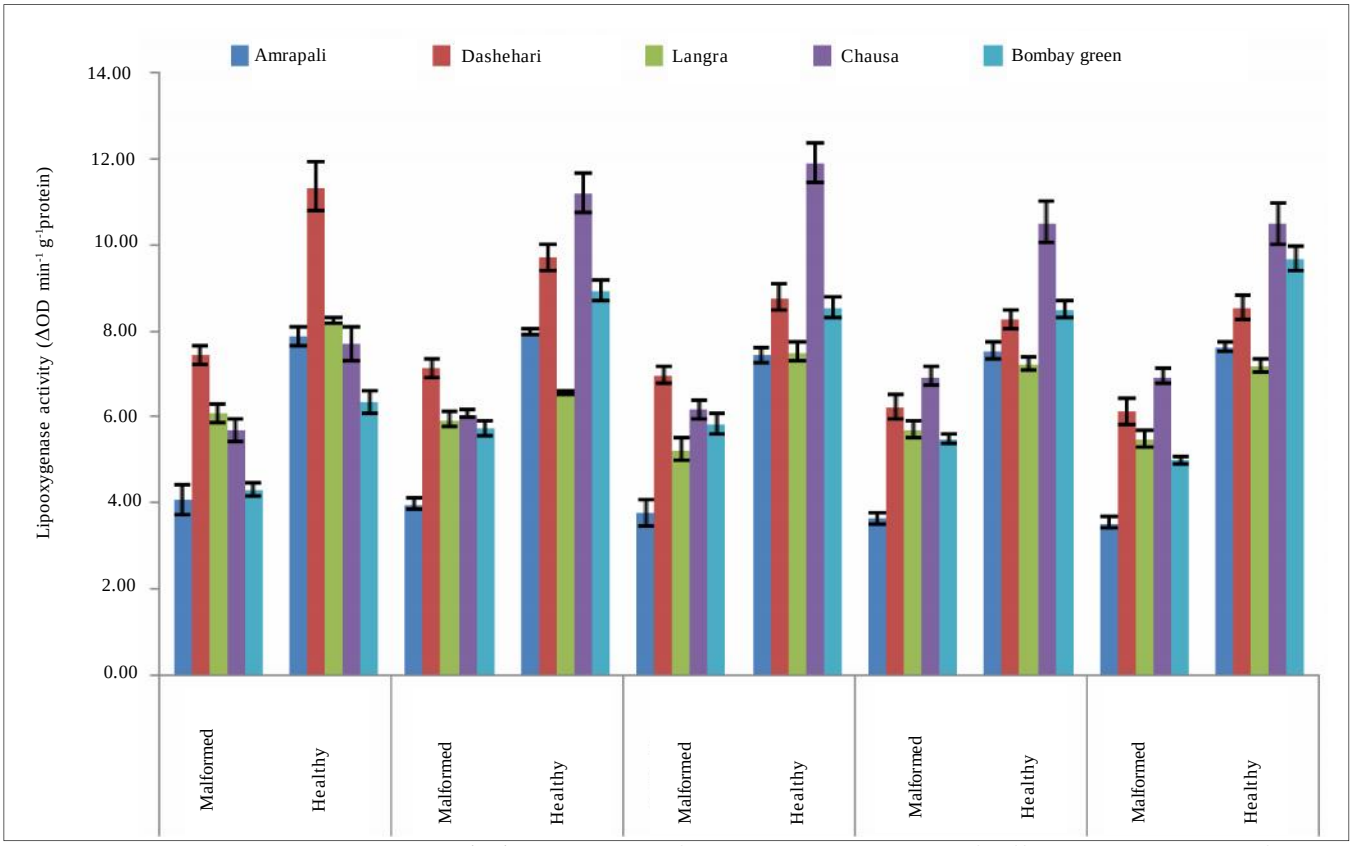

Fig. 3 : Lipooxygenase activity (D OD min^{-1} g^{-1} protein) in malformed and healthy leaves of different mango varieties from different states

Table 3: Lipooxygenase activity (aOD min^{-1} g^{-1} protein)in malformed and healthy leaves of different mango varieties from different states

Sr. No.	Variety	Lipoxygenase activity (Δ OD min^{-1} g^{-1} protein)									
		Bihar		Jharkhand		Uttar Pradesh		Uttarakhand		Delhi	
		Malformed	Healthy	Malformed	Healthy	Malformed	Healthy	Malformed	Healthy	Malformed	Healthy
1.	Amrapali	4.09 ±0.36	7.89 ±0.21	3.98 ±0.13	7.98 ±0.08	3.78 ±0.31	7.44 ±0.17	3.65 ±0.13	7.55 ±0.21	3.55 ±0.13	7.65 ±0.11
2.	Dasheri	7.44 ±0.20	11.36 ±0.58	7.14 ±0.20	9.72 ±0.31	6.99 ±0.20	8.78 ±0.31	6.23 ±0.29	8.27 ±0.22	6.13 ±0.29	8.54 ±0.28
3.	Langra	6.09 ±0.22	8.24 ±0.06	5.95 ±0.18	6.55 ±0.04	5.25 ±0.27	7.52 ±0.22	5.72 ±0.18	7.25 ±0.15	5.50 ±0.18	7.20 ±0.15
4.	Chausa	5.70 ±0.26	7.70 ±0.39	6.08 ±0.08	11.21 ±0.44	6.18 ±0.22	11.91 ±0.48	6.95 ±0.22	10.52 ±0.48	6.95 ±0.17	10.51 ±0.48
5.	Bombay green	4.32 ±0.16	6.35 ±0.25	5.75 ±0.18	8.95 ±0.25	5.85 ±0.23	8.55 ±0.25	5.50 ±0.09	8.50 ±0.20	5.00 ±0.09	9.69 ±0.29
	Mean	5.53	8.31	5.78	8.88	5.61	8.84	5.61	8.42	5.43	8.72
		Condition (A)	Variety (B)	Condition (A)	Variety (B)	Condition (A)	Variety (B)	Condition (A)	Variety (B)	Condition (A)	Variety (B)
	S.E.±	0.08	0.13	0.06	0.10	0.05	0.08	0.06	0.10	0.07	0.11
	C.D. (P=0.05)	0.26	0.41	0.20	0.32	0.16	0.25	0.19	0.30	0.21	0.33

Jharkhand, Uttar Pradesh, Uttarakhand and Delhi, respectively .

In a similar study the polyphenol oxidase was elevated in leaf tissues under salinity stress. The elevated polyphenol oxidase underneath stress indicates the potential to oxidize and degrade the toxic substance which include phenolic compounds that are usually collected all through salt stress. Under stressed environment the synthesis of polyphenol oxidase increase to minimize oxidative damage (Pollastri and Tittini, 2011).when *Phrgmites karka* was exposed to saline contition, the activity of polyphenol oxidase increases (Pirie *et al.*, 2013). However, increased polyphenol oxidase at higher salinity could not maintain a balance between ROS production and detoxification. This results in reduction of plant biomass.

Lipooxygenase activity (ΔOD min^{-1} mg protein^{-1}) in malformed and healthy leaf tissues of different mango varieties from different states :

Lipooxygenase activity in healthy and malformed tissues was estimated over two months (February-March) during flower initiation to flowering period in Amrapali, Dasheri, Langra, Chausa and Bombay green cultivars of mango in different states (Table 3 and Fig. 3).

It was apparent that lipooxygenase activity in malformed tissue was lower as compared to healthy tissue in all the cultivars in different states. Lipooxygenase activity in healthy vegetative tissue of Amrapali was 7.89, 7.98, 7.44, 7.55 and 7.65Δ OD min^{-1} mg protein^{-1} in Bihar, Jharkhand, Uttar Pradesh, Uttarakhand and Delhi, respectively and that in malformed vegetative tissue was 4.09, 3.98, 3.78, 3.65 and 3.55Δ OD min^{-1} mg protein^{-1} in Bihar, Jharkhand, Uttar Pradesh, Uttarakhand and Delhi, respectively.

Similarly in the healthy vegetative tissue of cultivar, Dasheri it was found to be 11.36, 9.72, 8.78, 8.27 and 8.54Δ OD min^{-1} mg protein^{-1} in Bihar, Jharkhand, Uttar Pradesh, Uttarakhand and Delhi, respectively and that in malformed vegetative tissue was 7.44, 7.14, 6.99, 6.23 and 6.13 Δ OD min^{-1} mg protein^{-1} in Bihar, Jharkhand, Uttar Pradesh, Uttarakhand and Delhi, respectively.

Similarly in the healthy vegetative tissue of cultivar, Langra it was found to be 8.24, 6.55, 7.52, 7.25 and 7.20Δ OD min^{-1} mg protein^{-1} in Bihar, Jharkhand, Uttar Pradesh, Uttarakhand and Delhi, respectively and that in malformed vegetative tissue was 6.09, 5.95, 5.25, 5.72 and 5.50Δ OD min^{-1} mg protein^{-1} in Bihar, Jharkhand, Uttar Pradesh, Uttarakhand and Delhi, respectively.

In the healthy vegetative tissue of cultivar, Chausa it was found to be 7.70, 11.21, 11.91, 10.52 and 10.51Δ OD min^{-1} mg protein^{-1} in Bihar, Jharkhand, Uttar Pradesh, Uttarakhand and Delhi, respectively and that in malformed vegetative tissue was 5.70, 6.08, 6.18, 6.95 and 6.95Δ OD min^{-1} mg protein^{-1} in Bihar, Jharkhand, Uttar Pradesh, Uttarakhand and Delhi, respectively.

In the healthy vegetative tissue of cultivar, Bombay green it was found to be 6.35, 8.95, 8.55, 8.50 and 9.69 Δ OD min^{-1} mg protein^{-1} in Bihar, Jharkhand, Uttar Pradesh, Uttarakhand and Delhi, respectively and that in malformed vegetative tissue was 4.32, 5.75, 5.85, 5.50 and 5.00 Δ OD min^{-1} mg protein^{-1} in Bihar, Jharkhand, Uttar Pradesh, Uttarakhand and Delhi, respectively.

Lipoxygenase activity increases during peroxidation of lipid membrane caused by ROS. However, there was occurrence of malformed and button berries in strawberry due to increase in lipoxygenase activity (Sharma and Singh, 2008). In finger millet, lipoxygenase oxidase play an important regulatory role in drought tolerance along with antioxidant enzymes. Lipoxygenase activity gradually increases during the progression of water deficit. It suggests that there is relationship of this enzyme with stress condition (Kotapati *et al.*, 2014).

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